

FORM PTO-1390
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

3190-010

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (if known see 37 CFR 1.5)

09/868953

INTERNATIONAL APPLICATION NO.
PCT/JP99/07152INTERNATIONAL FILING DATE
12/20/99PRIORITY DATE CLAIMED
12/22/98

TITLE OF INVENTION BONE RESORPTION INHIBITORS

APPLICANT(S) FOR DO/EO/US Kazuo SUZUKI, Satoshi YAMAGOE, and Tooru YAMAKAWA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 – 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
PCT/IPEA/409 (5 pages)
International Search Report (5 pages)

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/868953		INTERNATIONAL APPLICATION NO. PCT/JP99/07152		ATTORNEY'S DOCKET NUMBER 3190-010	
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21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445 (a)(2)) paid to USPTO \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY <div style="border: 1px solid black; height: 100px; width: 100%;"></div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 860.00 \$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	10 - 20 =	0	X \$18.00	\$ 0.00	
Independent claims	4 - 3 =	1	X \$80.00	\$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$.00	
TOTAL OF ABOVE CALCULATIONS =				\$1,070.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$ 0.00	
SUBTOTAL =				\$1,070.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.49(f)).				\$ 0.00	
TOTAL NATIONAL FEE =				\$ 80.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$ 0.00	
TOTAL FEES ENCLOSED =				\$1,070.00	
				Amount to be:	\$
				refunded	
				charged	\$

a. ☒ A check in the amount of \$ 1,070.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.

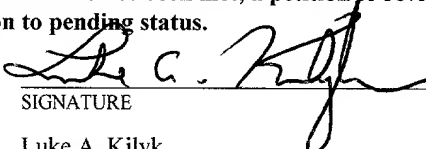
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0925. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

KILYK & BOWERSOX, P.L.L.C.
 53A Lee Street
 Warrenton, VA 20186


 SIGNATURE
 Luke A. Kilyk
 NAME
 33,251
 REGISTRATION NUMBER

09/868953

JC18 Rec'd PCT/PTO 2 2 JUN 2001

Date: June 22, 2001 Label No. EL856658299US

I hereby certify that, on the date indicated above, I deposited this paper or fee with the U.S. Postal Service and that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, DC 20231 by "Express Mail Post Office to Addressee" service.

Sandra Stocklinski

Sandra Stocklinski

Name (Print)

Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: SUZUKI et al.

)

Application No.: Unassigned

)

Parent Group Art Unit: Unassigned

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Filed: June 22, 2001

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Parent Examiner: Unassigned

)

For: BONE RESORPTION INHIBITORS

PRELIMINARY AMENDMENT

The Assistant Commissioner for Patents

Washington, D.C. 20231

June 22, 2001

Sir:

Prior to examination of the above-identified application on the merits, applicants respectfully request that the application be amended as follows:

IN THE TITLE:

Please delete the present title "BONE RESORPTION INHIBITORS ACTIVATING FACTOR OF LEUKOCYTES" and insert --BONE RESORPTION INHIBITORS--.

IN THE CLAIMS:

Please substitute the following amended claims for the pending claims with the same numbers in the above-identified application. (A version of the amended claims with markings to show the changes made is also attached.)

1. (Amended) A bone resorption inhibitor comprising leukocyte activating protein factor or leukocyte activating protein factor-derived substances, in an amount effective for bone resorption inhibitory activity.

3. (Amended) The bone resorption inhibitor according to claim 1, wherein the leukocyte activating protein factor or leukocyte activating protein factor-derived substances inhibits against osteoclast cell activity.

4. (Amended) The bone resorption inhibitor according to claim 1, wherein said substances have an inhibitory activity of more than 80% at a concentration of 10 µg/ml using percent inhibition of pit formation.

5. (Amended) A screening method for bone resorption inhibitor derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances, which are purified from the source of these substances, or which are prepared or synthesized by based on the information of these substances, comprising determining bone resorption inhibitory activity using percent inhibition of pit formation.

6. (Amended) A method to produce bone resorption inhibitors comprising introducing leukocyte activating protein factor or leukocyte activating protein factor-derived substances in the production of bone resorption inhibitors.

7. (Amended) A method for bone resorption inhibiting in an animal comprising administering to said animal, bone resorption inhibitor derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.

Please add the following new claims:

--8. The bone resorption inhibitor according to claim 2, wherein the leukocyte activating protein factor or leukocyte activating protein factor-derived substances inhibits against osteoclast cell activity.

9. The bone resorption inhibitor according to claim 2, wherein said substances have an inhibitory activity of more than 80% at a concentration of 10 µg/ml using percent inhibition of pit formation.

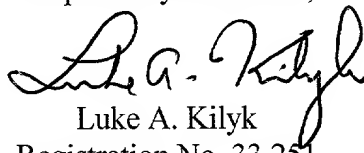
10. The bone resorption inhibitor according to claim 3, wherein said substances have an inhibitory activity of more than 80% at a concentration of 10 µg/ml using percent inhibition of pit formation.--

REMARKS

No questions of new matter are raised by the above amendment. Entry of the above amendment is therefore respectfully requested.

If there are any fees due in connection with the filing of this response, please charge the fees to deposit Account No. 50-0925. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to our Deposit Account.

Respectfully submitted,



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Attorney Docket No. 3190-010
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Warrenton, VA 20186
(540) 428-1701

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

1. (Amended) A bone resorption inhibitor comprising [which contains] leukocyte activating protein factor[-showing] or leukocyte activating protein factor-derived substances, in an amount effective for [activity to] bone resorption inhibitory activity.

3. (Amended) The bone resorption inhibitor according to claim 1 [or 2], wherein the leukocyte activating protein factor or leukocyte activating protein factor-derived substances inhibits against osteoclast cell activity.

4. (Amended) The bone resorption inhibitor according to claim 1 [any of claims 1-3], wherein said substances have an [where in an effective compound shows] inhibitory activity of more than 80% at a concentration of 10 µg/ml using percent inhibition of pit formation [in the screening methods pit formation, in candidate substances].

5. (Amended) A screening method [methods] for bone resorption inhibitor [inhibitors] derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances, which are purified from the source of these substances, or which are prepared or synthesized by based on the information of these substances, comprising determining bone resorption inhibitory activity using percent inhibition of pit formation.

6. (Amended) A method to produce bone resorption inhibitors comprising introducing [Use of] leukocyte activating protein factor or leukocyte activating protein factor-derived substances in the production of bone resorption inhibitors.

7. (Amended) A ~~method~~ [methods] for bone resorption inhibiting in ~~an animal comprising administering to said animal~~, [animals with] bone resorption inhibitor [inhibitors] derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.

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JC18 Rec'd PCT/PTC 22 JUN 2001

BONE RESORPTION INHIBITORS ACTIVATING FACTOR OF LEUKOCYTES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is concerned to a bone resorption inhibitor consisted of a leukocyte activating protein factor or a substance originating therein. A screening method against therapeutic for hypercalcemia, osteoporosis.

Description of the Prior Art

A leukocyte activating protein factor (hereinafter referred to LECT2 [Leukocyte-derived chemotaxinm 2]) has been found as a neutrophil chemotactic factor (Laid-open Patent publication No. Hei8-140683). Gene of human and bovine LECT2s have been cloned and their sequences have also been determined (S. Yamagoe et al., Immunol. Lett. 52, 9-13, 1996, S. Yamagoe et al., Biochem. Biophys. Acta, 1396, 105-113,, 1998). mRNA of human LECT2 codes 151 amino acids containing 18 amino acids sequence for its signal peptide. Amino acid sequences of ovine and human LECT2s show the higher homology to min-1 gene product derived from chicken. The min-1 product is contained in promyelocytes in the bone marrow and relates to generating control of oncogene myb, although its biological function has not been determined.

Action of LECT2, which was found as a chemotactic factor, is thought to use for diagnosis, therapy and follow up the diseases such as cancer, because increase of tumoricidal activity and production of interleukins from leukocytes due to activation of neutrophils with LECT2. Now, it is known that LECT2 only acts on neutrophils, action on bone metabolism has not been reported (Protein, Nucleic Acid and Enzyme-Tanpakushitu-Kakusan-Koso 42, 1086, 1997).

On the other hand, Fujio Suzuki et al. (Y. Hiraki et al., J. biol. Chem. 271, 22657-22662, 1996) isolated Chondromodulin-II, which is approximately 16 kDa protein from

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chondrocytes of fetal bovine. It is disclosed that Chondromodulin-II shows a promotion for proliferating chondrocytes and its differentiation (Laid open Patent publication No. H5-255398) and that chondromodulin has a promotion for proliferating osteoclast and has an act for activating osteoclast (Laid-open Patent Publication No. H8-27020). Recently, according to study on homology, it was found that Chondromodulin-II is the same as that of LECT2.

Osteoclasts, which are polynuclear large cells, act an adsorpting bone in bone tissues, thereby taking important part in re-modeling bone. Precursor cells will be derived from stem cells, and translocated on bone surface through circulated blood thereby being differentiated to osteoclast. On the other hand, osteoblast are differentiated from precursor cell, which belongs to stroma forming cells such as immature mesenchymal cells, fibroblasts and interstitial cell, and are derived from precursor of different cell line from those of osteoclasts. Bone forms by remodeling according to formation and resorption of bone repeatedly. Organizationally, remodeling of bone is carried out by resorpting, and, then, re-synthesizing bone from osteoblast, under reasonable balance. With aging, the balance change is occurred due to imbalance conditions of the metabolism, the weight of bone is decreased. With the continuation of this condition for long time, bone tissues get weak and cause osteoporosis, destruction of bone or pain in lumbar.

As agents against resorption of bone estrogen, calcitonin, bisphosphate and the like have been used, however side effects are also reported.

Accordingly, highly effective for inhibiting resorption of bone due to osteoclast and highly salty agents have been desired.

SUMMARY OF THE INVENTION

According to hard study, the inventors found the bone resorption inhibitor on osteoclasts, and eminently succeeded

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the invention.

An object of the present invention is to provide a novel bone resorption inhibitor.

Another object of the present invention is to provide the bone resorption inhibitor containing effective amount of substance derived from LECT2 or LECT2 derived substances.

Further object of the present invention is to provide the bone resorption inhibitor containing LECT2 or LECT2-derived substances involving sequences of amino acid number 1 to 151 or 19 to 151.

Still another object of the present invention is to provide the bone resorption inhibitor containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.

Still further object of the present invention is to provide screening methods for bone resorption inhibitors derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.

Still further object of the present invention is to provide the bone resorption inhibitors showing the inhibitory activity by more than 80% at a concentration of 10 $\mu\text{g/ml}$ in the screening methods pit formation mentioned above for bone resorption inhibitors in the candidate substances.

Still further object of the present invention is also concerned use of leukocyte activating protein factor or leukocyte activating protein factor-derived substances in the production of bone resorption inhibitors.

Still further object of the present invention is also concerned methods for treatment in animals with bone resorption inhibitors derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.

A main feature of the present invention is to find that LECT2 or LECT2-derived substances posses bone resorption inhibiting activity.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows results in the inhibition of bone resorption in unfractionated bone tissue cells with human LECT2 (hereinafter referred to as hLECT2) by the pit formation assay. Horizontal axis shows concentration of hLECT2 and vertical axis shows pit number/ivory slice. hLECT2 showed complete inhibitory activity (100%) at a concentration of 10 μ g/ml.

Figure 2 shows results in the inhibition of bone resorption in purified osteoclast cells with hLECT2 by the pit formation assay. As well as results in the assay using unfractionated bone tissue cells; hLECT2 showed completely inhibitory activity (100%) at a concentration of 10 μ g/ml using purified osteoclast cells.

DESCRIPTION OF THE PREFERRED EMBODYMENT

LECT2 in the invention can be purified as follows.

Purification of LECT2.

LECT2 in culture fluid of cells such as leukemic cells was concentrated with CM-Sepharose CL-6B (Pharmacia Biotech, Uppsala, Sweden) and DEAE-Sepharose (Pharmacia Biotech), CM-Sepharose CL-6B, hydroxylapatite and a reverse-phase column (Vydac C4 column 304-2151, 6 x x 250 mm) on HPLC. For example, leukocyte activating protein factor can also be purified from the culture fluid of SKW-3 leukemic cells stimulated with PHA for release of leukocyte activating protein factor into the fluid.

LECT2 or LECT2-derived substances can also be purified by a gene technology (Laid-open Patent publication No. Hei 8-140683, Hei 10-146189). For example, transformant cells may be produced with pMAL-TM-C or pGEX-3X as a vector. As host cells bacteria such as *E. coli*, yeast and animal cells may be used.

The animal cells, such as Chinese hamster CHO cells, monkey CVI cells, monkey CVI/293 cells, monkey COS cells, mouse fibroblast cells, mouse C127 cells, mouse 3T3 cells, mouse L-929 cells, human HeLa cells and human SKW-3 cells, which can express the recombinant plasmid encoding human LECT2, may be exemplified.

As regarding yeast, those established in a commercial production process, such as bread yeast, are convenient. In view of the industrial process, yeast-secreting line may be the most beneficially used.

Culture of these cells, purification of the protein in the invention from culture fluid, preparation of recombinant plasmid, and transformant cells, and usual purification of the protein from the transformant cells, can be used in a well-known manner.

LECT2-derived substances have inhibitory activity showing bone resorption, indicating the substance, is not specifically limited. The substances showing inhibitory activity of bone resorption are also involved in mutations such as deletion, replace, addition, and/or insertion on one or several sites in the amino acid (Ulmer, K.M., Science, 219, 666, 1983) of LECT2. Further, LECT2-derived substances are also involved in chimera protein, fusion protein, partial deletion protein, partial modified and chemically modified protein. Further, peptide or low molecular weight molecules preferred according to the primary-, secondary-, tertiary- and 3D-structures of LECT2 or LECT2-derived substances is also involved in the substances (Laid-open Patent Publication Nos. H5-255398 and H8-140683, WO/16177) (Li et al., Bioorganic & Medical Chemistry, 4, 1421-1427, 1996) (S. Yamagoe et al. B.B.R.C. 237, 116-120, 1997: Monoclonal antibody to a recombinant LECT2). Source of LECT2 or LECT2-derived substances is not restricted in species of animal if they have bone resorption inhibitory activity, but human is preferred for the antigenecity.

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In the present invention, the bone resorption inhibitory activity of LECT2 or LECT2-derived substances can be assayed for example as follows. Bone resorption inhibitory activity of LECT2 or LECT2-derived substances is assayed by the inhibition of pit formation during incubation overnight by osteoclast cells isolated from rabbit are placed on ivory slice (Takeda et al. Bone and Mineral 17, 347-359, 1992) (Kameda, et al, Nihon Ykuri Zasshi 109, 74-84, 1997). Substances showing bone resorption inhibitory activity can be selected by the screening using percent inhibition of pit formation. For example, the substance showing 80% of present inhibition under concentration of 10µg/ml may be selected.

As bone diseases osteoporosis for use of substances having bone resorption inhibitory activity mentioned in the invention, hypercalcemia, hyperparathyroid and Bechet are listed.

When use of substances having bone resorption inhibitory activity mentioned in the present invention, 0.005-10 mg/kg or 0.01-3 mg/kg in preferentially with separation into 3 times in the dosage may be made, and the dosage can be increased or decreased from the recommendation according to chemical character, disease state and age and etc.

Constituents of pharmaceutical compositions, in addition to the active agents described herein, include those generally known in the art for the various administration methods used. For example, oral forms generally include powders, tablets, pills, capsules, lozenges and liquids. Similarly, intravenous, intraperitoneal or intramuscular formulations will generally be dissolved or suspended in a pharmaceutically acceptable carrier, e.g., water, buffered water, saline and the like. Additionally, these compositions may include additional constituents which may be required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity

adjusting agents, wetting agents and the like. For solid compositions, conventional nontoxic solid carriers may be used which include, e.g., pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate and the like.

According to the present invention, it was found that LEC2 contained the inhibitory activity against bone resorption, and therapeutic treatment for hypercalcemia and osteoporosis etc. could be presented. Further, the screening method to get bone resorption substance could be also presented.

EXAMPLES

The present invention is further explained by way of example as follows.

This is an example for practical use; the invention is not restricted by this example.

Example 1

(1) Unfractionated cells

Infant rabbit about 110g 10 day-old was sacrificed under diethyl ether as anesthetics. After removal of soft tissues, bone from 4 legs was isolated. And then the bone was minced in a α -minimal essential medium (α -MEM) containing 5% fetal bovine serum (FBS). The sufficiently minced bone and the medium was mixed with Vortex mixer to remove cells stacked on the bone. After 2 minutes, the unfractionated cells in the supernatant were collected.

(2) Pit assay

Ivory slice was prepared by cutting ivory into the disk-form piece (6 mm in diameter) with 20-40 μ m in depth, and then sterilized with ultrasonic treatment of 70% ethanol. After each ivory slice was washed with phosphate buffered saline (PBS) and alpha-MEM medium, it was transferred to 96-well plate with

200 μ l of culture medium containing α -MEM and 5% FBS. After incubation of the plate in a CO₂ incubator (5%CO₂ and 96%air) for 2 hrs at 37°C, the culture medium was removed from the well completely, culture medium containing hLECT2 at a several concentrations and 5×10^5 cells of unfractionated bone cells were added to the well. After incubation again under the same conditions for 18 hours, cells attached on the ivory slice were completely removed with rubber polisher. Then, the slice was stained with acidic hematoxyline solution for few minutes at room temperature. Bone resorption activity in the slice was measured by number of pit with a microscopic observation.

Figure 1 shows results in inhibition of bone resorption in unfractionated bone tissue cells with human LECT2 (hLECT2) by the pit formation assay. Horizontal axis shows concentration of hLECT2 and vertical axis shows pit number/ivory slice. hLECT2 showed complete inhibitory activity at a concentration of 10 μ g/ml.

Example 2

(1) Purification of osteoclast cells

The unfractionated cells obtained in the Example 1 were plated into a plastic dish, which were coated with collergen gel (Nitta Zeratinin Cell Matrix Type I, Co., Tokyo, Japan) the supernatant were collected. After incubation of the plate in a CO₂ incubator (5%CO₂ and 95%air) for 2 hrs at 37°C, the culture medium was removed from the well completely, the dish was washed with PBS three times to remove the cells on the gel. Then, the dish was washed with PBS containing 0.01% pronase E and 0.02% EDTA solution three times again. Cells without osteoclast were completely removed with the incubation for 5 minutes at room temperature in PBS containing collagenase. The remained cells containing attached molecules on the gel in the dish were collected after adding PBS containing 0.1% collagenase and

standing for 10 minutes at room temperature to obtain cell suspending solution in which osteoclast was exclusively contained.

(2) Pit assay

Pit assay was preformed using the ivory slice and the culture medium by the same procedures as that described in the Example 1 with exception for use of 3000 purified osteoclast cells in a well, instead of the unfractionated bone cells. Figure 2 shows results in inhibition of bone resorption in purified osteoclast cells with human LECT2 (hLECT2) by the pit formation assay. As well as results in the assay using unfractionated bone tissue cells; hLECT2 showed completely inhibitory activity at a concentration of 10 $\mu\text{g/ml}$ using purified osteoclast cells.

Example 3

(Acute toxicity)

Substances having sequences of amino acid number 19 to 151 of the leukocyte activating protein factor prepared by the well-known procedures were injected into venous of 5 ddY mice (body weight 20 ± 1 g) at dosage 0.1mg/g.

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What is claimed is:

1. A bone resorption inhibitor which contains leukocyte activating protein factor-showing or leukocyte activating protein factor-derived substances, in an amount effective activity to bone resorption inhibitory.
2. The bone resorption inhibitor according to claim 1, where in the leukocyte activating protein factor has sequences of amino acid number 1 to 151 or 19 to 151.
3. The bone resorption inhibitor according to claim 1 or 2, wherein the leukocyte activating protein factor or leukocyte activating protein factor-derived substances inhibits against osteoclast cell activity.
4. The bone resorption inhibitor according to any of claims 1-3, where in an effective compound shows inhibitory activity of more than 80% at a concentration of 10 $\mu\text{g/ml}$ in the screening methods pit formation, in candidate substances.
5. A screening methods for bone resorption inhibitors derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances, which are purified from the source of these substances, or which are prepared or synthesized by based on the information of these substances.
6. Use of leukocyte activating protein factor or leukocyte activating protein factor-derived substances in the production of bone resorption inhibitors.
7. A methods for bone resorption inhibiting in animals with bone resorption inhibitors derived substances containing leukocyte activating protein factor or leukocyte activating protein factor-derived substances.



(51) 国際特許分類7 A61K 38/17	A1	(11) 国際公開番号 WO00/37093 (43) 国際公開日 2000年6月29日(29.06.00)
(21) 国際出願番号 PCT/JP99/07152 (22) 国際出願日 1999年12月20日(20.12.99) (30) 優先権データ 特願平10/363727 1998年12月22日(22.12.98) JP (71) 出願人 (米国を除くすべての指定国について) 国立感染症研究所 (NATIONAL INSTITUTE OF INFECTIOUS DISEASES) [JP/JP] 〒162-8640 東京都新宿区戸山1丁目23番1号 Tokyo, (JP) (71) 出願人; および (72) 発明者 鈴木和男(SUZUKI, Kazuo)[JP/JP] 〒299-4501 千葉県夷隅郡岬町椎木633番地2 Chiba, (JP) (72) 発明者; および (75) 発明者/出願人 (米国についてののみ) 山越 智(YAMAGOE, Satoshi)[JP/JP] 〒277-0084 千葉県柏市新柏1丁目18番地 =新柏住宅4棟102号室 Chiba, (JP)	山川 徹(YAMAKAWA, Tooru)[JP/JP] 〒160-8515 東京都新宿区四谷1丁目7番地 持田製薬株式会社内 Tokyo, (JP) (74) 代理人 庄司 隆, 外(SHOJI, Takashi et al.) 〒101-0032 東京都千代田区岩本町3丁目9番9号 第一瀬野ビル1階 Tokyo, (JP) (81) 指定国 CA, US, 欧州特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE) 添付公開書類 添付公開書類 国際調査報告書 (88) 国際調査報告書の公開日: 2000年11月2日(02.11.00)	
(54) Title: <u>BONE RESORPTION INHIBITORS</u> (54) 発明の名称 骨吸収抑制剤 (57) Abstract A leukocyte activating protein factor or a substance originating therein having an effect of inhibiting bone resorption; and novel medicinal utilization thereof. Use of these novel substances with the bone resorption inhibitory effect makes it possible to provide therapeutic methods efficacious against hypercalcemia, osteoporosis, etc.		

Fig. 1

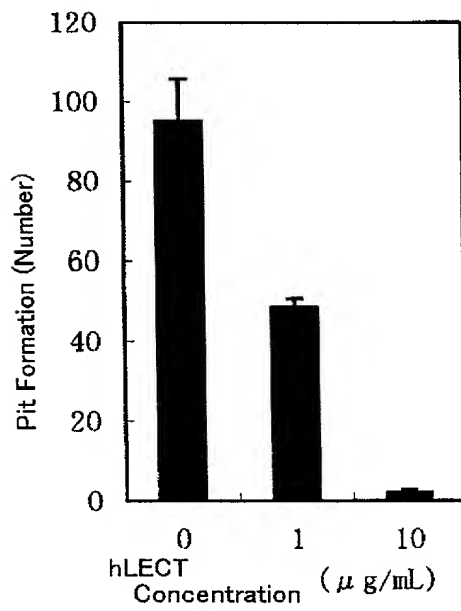
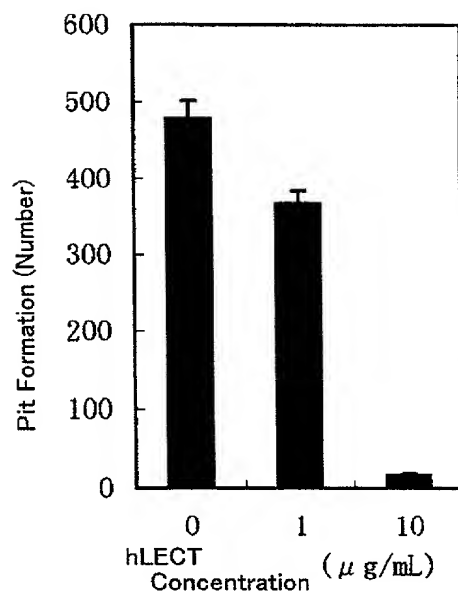


Fig. 2





FP99-1015/US

Docket No. 3190-010

KILYK & BOWERSOX, P.L.L.C.

Declaration for U.S. Patent Application

As a below named inventor, We/I hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names.

We/I believe We/I are the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **BONE RESORPTION INHIBITORS**, the application of which is attached hereto unless the following is checked

- ☐ was filed on _____, as United States Application Number _____ and
☐ was amended on _____ (if applicable).

We/I hereby state that We/I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

We/I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

We/I hereby claim foreign priority benefits under Title 35, United States Code, § 119 (a) - (d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application for which priority is claimed:

(List prior foreign applications.)	<u>10/363727</u> (Number)	<u>Japan</u> (Country)	<u>22 Dec. 1998</u> (Day/Month/Year Filed)	Priority Claimed <u>x</u> Yes ___ No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	___ Yes ___ No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	___ Yes ___ No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	___ Yes ___ No

___ See attached list for additional prior foreign applications

We/I hereby claim the benefit under Title 35, United States Code, § 120 or § 119(e) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, We/I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(List Prior U.S. Applications)	<u>PCT/IP99/07152</u> (Appln. Serial No.)	<u>20 Dec. 1999</u> (Filing Date)	<u>Pending</u> (Status: Patented, Pending, Abandoned)
	_____ (Appln. Serial No.)	_____ (Filing Date)	_____ (Status: Patented, Pending, Abandoned)
	_____ (Appln. Serial No.)	_____ (Filing Date)	_____ (Status: Patented, Pending, Abandoned)

We/I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Luke A. Kilyk, Reg. No. 33,251; and Leonard D. Bowersox, Reg. No. 33,226

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We/I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the United States Code, § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See note C above)

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Full name of fourth inventor (given name, family name) _____

Inventor's signature _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

Sequence Listing

<110> National Institute of Infectious Disease

Kazuo Suzuki

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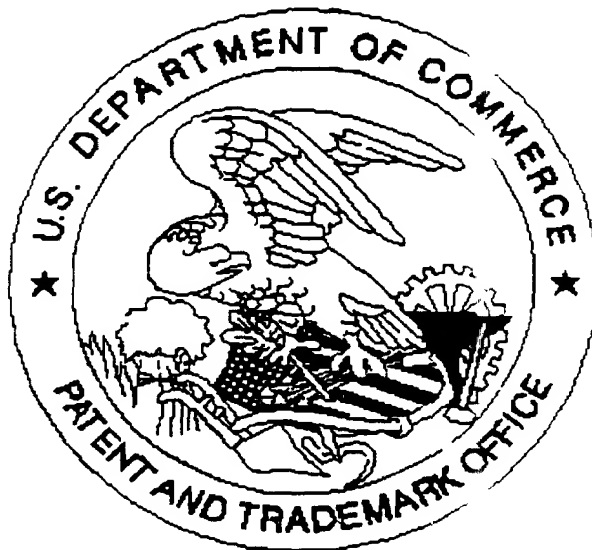
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*There is only 1 sheet of drawings with
two figures on it. And 2 sheets of sequence
listing number 1 and 2.*

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